XXVIII. CEREALS AND RICKETS.

VI. THE COMPARATIVE RICKETS-PRODUCING PROPERTIES OF DIFFERENT CEREALS.[1]

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UP to 1921 little attention had been given to the variation in the rickets-producing capacity of different cereals or cereal products. E. Mellanby [1921], working with dogs, reported a wide variation in this respect between cereals, particularly between oatmeal and white flour. Later [1925] he reported additional data in support of his earlier findings and offered a hypothesis to explain the observed differences. At this time our experience with rats and dogs had impressed us with the similarity of cereals in the production of mild rickets and we reported briefly [Steenbock *et al.* 1927] these preliminary observations. Later Green and E. Mellanby [1928] reported a confirmation of Mellanby's earlier conclusions; this time also employing the rat as the experimental animal.

So far as we are aware, E. Mellanby and co-workers, as quoted above, M. Mellanby [1928; 1929; 1930], Holst [1927], Mirvish [1929; 1930], Mirvish and Bosman [1929], Christiansen [1934], and Steenbock *et al.* [1927; 1930] have compared various cereals or cereal products for calcification of bone or teeth. In contrasting oatmeal with other cereals and their by-products, E. Mellanby [1925] referring to conditions in England states, "Apart from extreme malnutrition, however, it would appear not improbable that in this country, where the average diet is either deficient in or contains a border-line quantity of antirachitic vitamin and calcium, and where sunshine is negligible, the ingestion of oatmeal during pregnancy and lactation of women, and in growing children, does much harm."

If Mellanby's contentions be true, then in view of the prominent place which cereals and their by-products occupy in both human and livestock nutrition, more attention should be given to the comparative study of the specific rickets-producing properties of various cereals in spite of the availability of efficient corrective measures. In this paper we are reporting three series of experiments using the low calcium diets of E. Mellanby, which serve as a check on our earlier conclusions.

EXPERIMENTAL.

The experiments of Series I were carried out with four cereals, *viz.* yellow maize, rolled oats,[3] whole wheat and patent flour. To eliminate variations in consumption of ration as a determining factor in the outcome of the experiment we equalised the food intake. In each case the consumption was so adjusted that it

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[3] A form of oatmeal representing at least 95 % of all oatmeal used for human consumption in the U.S.A.

was always limited by the least amount consumed by any rat in the various groups, except when that rat showed obvious abnormalities due to other causes than those attributable to the ration. We have used this technique of "equalised consumption" for the last twelve years and consider it indispensable under certain conditions. Mitchell and Beadles [1929] have adopted a similar technique as "paired feeding".

When the nature of the results of this first series of experiments was becoming evident, Series II was started with the same cereals except for the addition of polished rice. This series was run to determine whether the obvious variations from Green and E. Mellanby's [1928] results might not be explained by the fact that they did not secure accurate control of consumption. Green and Mellanby state that they fed 7·5 g. of the cereal and 2·5 g. of the supplement daily, but at the same time make it clear that in the early part of their experiments the animals did not always consume the amount offered. It also is very obvious that with animals of the weight and size used by them it would have been exceedingly difficult, if not impossible, to secure the 10 g. daily consumption. Neither do Green and Mellanby state how much was left unconsumed, and therefore the reader is unable to come to any definite conclusions, nor is he in position to evaluate the variations. To determine the variations which might have been brought about by their technique, we practised *ad libitum* feeding in this series but at the same time kept careful records of the amounts consumed from day to day.

Series III was started, again using the technique of "equalised consumption". Here also five cereals were used, the same as in Series II. Except for the one additional cereal, *viz.* polished rice, this series therefore represents a duplication of the first one.

All the rats used for the experiments were of a piebald closely inbred stock of the same ancestry as that which has been used in our laboratory for the last twenty years. They were raised on our stock ration [Steenbock, 1923] slightly modified to the extent that 5 % of butter fat was mixed in with the grain ration to provide a sufficient excess of vitamin A so as to make possible continuous breeding without rest periods after weaning of the young. With this addition of butter fat we have never observed deficiencies of vitamin A in the stock ration. Nor have we ever noticed that the addition of this amount of butter fat introduces sufficient vitamin D appreciably to affect the storage reserves of the young.[1]

Six animals were put on each cereal except in Series I where only four were used. In order to equalise as much as possible the effect of litter variations in the development of bone, each group of rats kept on a cereal had apportioned to it a representative from each of the litters used. Furthermore, an attempt was made to secure the same number of the two sexes in each group, because it is a well-known fact, first observed by Hammett [1925], that females uniformly have a slightly higher ash content in their bones than males. In Series I there were three females to one male for each cereal; in Series II, 3 females to 3 males, and in Series III, 2 females to 4 males. In all cases only litters approximating to 60 g. in weight per rat at an age of approximately 25 days were used. The actual weights taking the series in order I to III were as follows: 58·9, 58·2 and 58·4 g. with ranges in each series of 55 to 63, 54 to 62 and 55 to 64 g., respectively. The average ages were 24, 23·9 and 26·7 days for Series I, II and III respectively, with ranges in each of 23 to 25, 22 to 26 and 26 to 29 days, respectively. The rats were confined individually in rectangular cages measuring $10 \times 10 \times 20$ in. Each cage was provided with an elevated false screen bottom of large mesh.

[1] The results with this ration can be markedly improved by the supplemental feeding of "greens" *ad libitum* two to three times a week.

In the experiments already published on rickets we have depended for the most part upon the use of the 3143 ration of McCollum, Simmonds, Shipley and Park [1922, 3] or the 2965 ration of Steenbock and Black [1925] for the production of rickets. In the present series of experiments we used the same ration formulae as those used by Green and E. Mellanby [1928]. The cereals were finely ground in a ball mill. It has been our general experience that cereals of a hard and flinty nature, such as rice and maize, tend to reduce consumption in young rats undoubtedly owing to difference in their physical texture, because we have not experienced this when they were finely ground.

All our rations as in the experiments of Green and E. Mellanby [1928] were fed uncooked. As supplements to the cereals we used a basal mixture of marmite 32 g., caseinogen 24 g., cabbage 25 g., lemon juice 32 g. and sodium chloride 12 g. One part of this was used with 3 parts of cereal. The marmite was furnished in the form of "Vegex". We were assured by the manufacturers that this product was identical with marmite except for the fact that it contained somewhat larger quantities of sodium chloride. The caseinogen was furnished in the form of commercial "casein", which we extracted repeatedly with dilute hydrochloric acid to reduce its content of minerals, particularly calcium. The use of the purified caseinogen we considered especially important in comparing cereals for rickets production, because E. Mellanby [1925] and Green and E. Mellanby [1928] emphasised repeatedly that the differences in the anticalcifying properties of the various cereals are particularly evident on a low intake of calcium in the absence of vitamin D. The dried cabbage represented shredded cabbage, from heads which had been cored and freed almost entirely from green leaves, dried at a temperature of 55° overnight. After drying it was finely ground for later mixing with the other constituents.

The various supplements, namely the marmite ("Vegex"), caseinogen, cabbage, lemon juice and in addition sodium chloride, were mixed in small quantities at intervals of 3 or 4 days. This intermittent mixing of the supplement, though not the most desirable procedure, was followed because the marmite was of salve-like consistency and possibly could have lost some of its original nutritional value by drying and exposing to the atmosphere. This mixture was weighed out in portions equal in number to the various cereals fed, and each portion remixed with a cereal in the proportion of 1 supplement to 3 cereal. Equal portions of the final mixtures were then weighed out daily for each rat in the various cereal groups. The daily portions were fed in small tin cups. At the close of the experiments, the accumulated spillage observed in the bottom of the pans was found to be negligible.

In making up the rations as above outlined, we made every attempt to duplicate the Green and E. Mellanby [1928] technique from their description. It must, of course, be emphasised that it was impossible to duplicate their conditions exactly. In the first place, cereal grains and their products are not constant in composition. In the second place, it is very obvious that the cabbage and lemon juice as well as caseinogen and marmite, were somewhat different in composition unless mere chance should have made them the same. It is, furthermore, possible that the cabbage may in one or the other case have contained some vitamin D. We sought to remove this possibility in our experiments by discarding the outside green leaves of the heads. As to whether this was done by Green and E. Mellanby we were unable to determine from their paper. The cereals in our experiments, with the exception of the yellow maize in Series I, were taken from one source. The supplements in Series II and III were the same but of different origin from those used in Series I. The lemon juice, being

extracted from fresh lemons every third or fourth day, was, of course, different in each series.

The animals were weighed weekly, at which times detailed notes were made on their physiological condition. These included not only the observed tendencies to reduced food consumption, but also their general activity and incidence of ophthalmia. It is to be noted that in none of these experiments were there ever observed the slightest signs of vitamin A deficiency. All the experiments were terminated after five weeks. The animals were anaesthetised with ether and bled from their carotids by the technique of Bethke *et al.* [1923] for pooled samples of blood. The bloods were kept in a refrigerator overnight, then centrifuged and the sera used for calcium and inorganic phosphorus determinations according to the techniques of the Clark-Collip [1925] modification of the Tisdall method and the Briggs [1922] modification of the Bell-Doisy method respectively. The wrist bones were dissected free and then preserved in 10 % formalin for later examination of the widening of the epiphyseal line as occurs in rickets. For this we used the technique developed in the Johns Hopkins laboratories [McCollum, Simmonds, Shipley and Park, 1922, 3] except for the fact that our observations were made only in gross without the use of a microscope. The ribs were also preserved in 10 % formalin for later macroscopic comparison as to degree of involvement of the costochondral junctions. The freshly dissected and cleaned femora were collectively and continuously extracted with alcohol for a period of five days in a Soxhlet apparatus. They were then dried, weighed and ashed in an electric muffle furnace for determination of their ash content. We used this technique of ash analysis after Dibbelt [1909] had pointed out that variations in the lipoid content of bones brought about big variations in the percentage of ash unless such lipoids were first removed.

Food consumption.

The average data on consumption of rations are shown in Table I. We consider these data as accurate as it is possible to obtain them in experiments with small animals. In Series I the consumption was equalised within 2 g. per rat in

Table I. *Food consumption.*

Per rat in five weeks.

Cereal additions	Average total intake (g.)			Range of total intakes (g.)		
	I*	II†	III*	I*	II†	III*
Yellow maize	242	281	218	All 242	242–305	All 218
Rolled oats	242	234	217	240–244	190–265	209–221
Whole wheat	241	285	216	241–242	224–391	205–218
Patent flour	240	250	218	237–242	215–289	216–218
Polished rice	—	235	213	—	203–262	201–220

* Equalised consumption. † *Ad libitum*, recorded consumption.

each group for the entire period of five weeks, with a maximum range of 7 g. between all the rats of the series. In Series III the consumption was equalised within 5 g. per rat in the five-week period, with a maximum range of 20 g. between all the rats in the series, with the exception of one. This rat, No. 816, ate only 160 g. instead of the approximate average of 200 g.; it therefore, being obviously abnormal, was omitted from the calculations. In Series II, which was run with *ad libitum* consumption, the maximum variation was much larger. It amounted to 51 g. as the average between the groups, with a maximum range between all individuals in the series of 201 g.

The different rations were not always consumed with the same facility in each series. In Series I, the consumption of rolled oats was the most limiting factor; patent flour next and yellow maize the least. In Series II polished rice and rolled oats were consumed the worst, with yellow maize and whole wheat the best. In Series III polished rice was again the most limiting, with rolled oats next in order and yellow maize the least.

From certain points of view, the value of attempting to equalise consumption may be questioned. It is a well-known fact after the observations of McCollum and co-workers that the severity of rickets produced on a rachitic diet is increased by the amount of ration consumed, but at the same time it is not clear whether this increased severity of rickets is due to the fact that the greater consumption of food causes greater growth and therefore greater impoverishment of bone in calcium and phosphorus; or whether the greater growth is the responsible factor and greater consumption of ration merely follows as an effect. E. Mellanby and Green have repeatedly pointed out how increased consumption of cereal increases the severity of rickets [1921; 1925; 1928], but in their latest paper they apparently placed more emphasis upon maintaining uniformity of growth than upon maintaining uniformity of consumption of ration.

In analysing these results, Green and E. Mellanby [1928] state that it was very difficult to obtain uniformity of growth because after the first few weeks the animals varied decidedly in their appetites. As examples of variation in weight, in Table I, the maximum weights attained varied from 47 to 94 g.; in Table II, for Series I they ranged from 66 to 106 g., and for Series II, 68 to 96 g.; in Table III from 51 to 81 g.; in Table IV from 50 to 113 g.; in Table V from 47 to 100 g. *etc.* From their data we are therefore forced to conclude that Green and E. Mellanby [1928] accomplished neither equalised consumption nor equalised growth. We, ourselves, are of the opinion that whatever may be the fault of limiting our experiments to equalised consumption, inasmuch as we were successful in accomplishing this purpose, our data put us in the position to evaluate this technique and later to extend our endeavours to secure rigid control over growth as well. Though this is admittedly difficult, if it is successfully accomplished, we shall then be able to compare the two factors.

Growth.

Our present data on growth obtained by taking increases in body weight are presented in Table II. We are not entirely satisfied with this technique because

Table II. *Summary of average growth values.*

	Rat weights										
	Series I				Series II				Series III			
	Weights (g.)			Maximum increase %	Weights (g.)			Maximum increase %	Weights (g.)			Maximum increase %
Cereal additions	Initial	Maximum	Final		Initial	Maximum	Final		Initial	Maximum	Final	
Yellow maize	59·2	95·0	95·0	60·3	57·8	99·7	99·7	72·5	57·2	79·2	75·5	38·5
Rolled oats	60·0	104·0	104·0	73·3	58·5	88·5	87·7	51·3	58·5	78·5	75·8	34·2
Whole wheat	59·0	91·8	91·8	55·6	58·2	98·5	98·5	69·2	58·8	79·0	74·7	34·4
Patent flour	57·2	92·5	92·5	61·7	58·2	87·3	87·0	50·0	58·0	81·7	76·3	40·9
Polished rice	Not included in Series I				58·3	91·0	91·0	56·1	59·3	84·0	83·8	41·7

it assumes that the increase in weight represents symmetrical body growth which is not necessarily the case, but we accept it for the time being. It has already been mentioned that all rats in the series, inasmuch as there were litter representatives in each group, have the same average ages for the group. The initial average weights, for all practical purposes, were also the same. Without going into detail, the differences in average initial weight between groups were 2·8 g. for Series I, 0·7 g. for Series II and 2·1 g. for Series III. This we believe represents an accurate measure of the uniformity of our rats as used for the different cereal experiments. This also stands in marked contrast to the animals used by Green and E. Mellanby [1928] who, as their tables show, used animals weighing from 29 to 52 g. in their various experiments in which they compared one cereal with another. They also made no statements in regard to initial ages of their animals.

Although, as stated before, we made no direct attempt to obtain uniformity of growth, this was practically accomplished unintentionally as seen by inspection of the table for maximum weights attained and for percentages of resultant increases in weight. In Series I the average maximum weights range from 91·8 g. for whole wheat as the minimum to 104 g. for rolled oats as the maximum. This gives percentages of increase of 55·6 and 73·3 respectively. In Series III the range of average maximum weights was from 78·5 g. for rolled oats as the minimum to 84·0 g. for polished rice as the maximum. Calculated as percentages of increase this gives values of 34·2 to 41·7 respectively. The resultant growth for Series II (*ad libitum* consumption) showed no marked difference from those obtained in Series I and III; the average maximum weights ranged from 87·3 g. for patent flour to 99·7 g. for yellow maize, giving a range of percentage increase of 50·0 to 72·5. It is to be added that such comparable results were unexpected because originally we had anticipated that Green and E. Mellanby's results might be due to differences in consumption of ration secured with their *ad libitum* technique.

The range of difference of percentage increase in weight was greater in the experiments of Green and E. Mellanby than in our own. For instance, in Table II under Series I their increases range from 100 % in the case of oatmeal to 186 % with white flour. In Series II they obtained increases in weight from 112 % in the case of rice to 217 % with white flour, and in Table III from 10 % with rice to 145 % with white flour. They point out that in the majority of cases in which rice was used, growth was so poor that it did not allow a good comparison with other cereals.

Green and E. Mellanby emphasised that experiments with rickets-producing cereals should be ended, if possible, during the period of growth and not prolonged when the animals have begun to lose weight. We agree with them that this is a desideratum. We practically accomplished what they considered essential, except for Series III. There we did encounter some losses in weight. These losses, when averaged for the different rats, amounted to only 4·2 g. We have no way of knowing whether or not Green and E. Mellanby came closer to meeting the above desirable conditions, because they presented only maximum weights in their tables. The difficulty that they experienced in securing consumption with rice, resulting in a maximum weight increase in one case of only 10 % above the initial weight of the animals in 30 days, leads us to suspect that they had larger losses because in our experiment we obtained far better performance. We are unable to account for the losses in weight experienced by our rats in Series III, but whatever their origin our results are not influenced by it *in toto* because in Series I and II increases in weight were observed to the end.

Epiphyses and costochondral junctions.

The comparative degree of rachitic involvement of the epiphyses of the distal ends of the radii and ulnae, and of the costo-chondral junctions are presented in Table III. Numbers from 1 to 5 are used to indicate the progressive severity of

Table III. *Epiphyses and costo-chondral junctions (individual records).*

Cereal additions	Epiphyses*			Costochondral junctions†		
	I	II	III	I	II	III
Yellow maize	1 | 1	4 | 1	1 | 1	0 | 0	0 | 0	0 | 0
	2 | 1	3 | 3	1 | 1	3 | 0	0 | 0	0 | 0
		3 | 3	1 | 2		0 | 0	0 | 3
Rolled oats	1 | 1	1 | 1	0 | 1	0 | 0	0 | 0	0 | 0
	1 | 1	1 | 1	1 | 1	0 | 0	0 | 0	3 | 0
		0 | 1	1 | 1		0 | 0	0 | 0
Whole wheat	1 | 1	1 | 0	0 | 1	0 | 0	0 | 0	3 | 3
	1 | 1	0 | 1	1 | 1	0 | 0	3 | 3	0 | 0
		1 | 2	1 | 1		0 | 0	0 | 0
Patent flour	2 | 1	1 | 2	1 | 1	3 | 0	0 | 0	0 | 0
	2 | 1	1 | 0	1 | 1	3 | 0	0 | 0	0 | 3
		1 | 1	1 | 1		0 | 0	3 | 0
Polished rice	— —	1 | 1	1 | 2	— —	0 | 0	3 | 0
		1 | 1	1 | 2		0 | 0	3 | 0
		1 | 1	1 | 1		0 | 0	3 | 0

* Condition of epiphyses: 0 denotes normal, 1 denotes narrow rachitic, 2 denotes medium rachitic, 3 denotes medium to wide rachitic, 4 denotes wide rachitic, 5 denotes very wide rachitic.

† Conditions of costo-chondral junctions: 0 denotes normal, 3 denotes moderate beading, 5 denotes very marked beading.

the lesions with 0 for the normal. In general the rickets produced was not of a severe type because it did not exceed stage 3, which represents only a moderate rickets. The yellow maize group in Series II stands out from the others in having one animal in stage 4 and more than any others in stage 3, but the costo-chondral junctions in this group were all normal. It may be assumed that variations in beading can result from differences in behaviour of individuals with resultant variations in strain and hyperplasia of the rib junctions. Possibly it may also be granted that the widths of the metaphyses are determined by changes in the rapidity of growth from time to time as the deposition of mineral elements and concomitant growth of ossein fails to keep pace. We have noted, for instance, that with cessation of growth in severe rickets the metaphyses do not increase in width even though the rachitic condition as judged by other criteria becomes more severe. It is noteworthy that the average growth on the yellow maize in the aforementioned instance was greater during the last week than in other serial groups.

Femora.

In a comparative macroscopic study of the severity of rickets, probably the most significant data to consider are those concerned with the size of the bones and their mineralisation. In this respect we have employed the alcohol-extracted dried weight, the total ash weight, and the percentage of ash in the femora. The values of percentage of ash contained in the femora were calculated on the basis of their alcohol-extracted dried weights. Green and E. Mellanby [1928] used as indices the percentage of calcium in the fat-extracted dried bones, and the A/R ratio, where A represents the weight of bone ash and R the difference between the weights of the fat-extracted dried bone and the ash. In our experience with the data of these experiments and those of Green and E. Mellanby, a very high positive correlation was found to exist between the percentage of calcium, percentage of ash, and the A/R ratio. Consequently, each may serve equally well as a measure of the degree of rachitic involvement.

In Table IV we have reported the data for Series I. Attention is called particularly to weight of the total ash and percentage of ash in the femora. These data are typical of the ranges of variation shown in all three series. We have

Table IV. *Detailed data of Series I on femur analyses.*

Rat no.	Initial weight and sex g.	Total gain g.	Average consumption daily g.	Femora		
				Dry weight mg.	Ash weight mg.	% ash
			Yellow maize			
544 a*	62 ♂	36	6·9	117·1	51·4	43·9
545 b	63 ♀	32	6·9	115·1	52·3	45·5
546 c	55 ♀	43	6·9	121·7	55·7	45·8
547 d	57 ♀	32	6·9	111·4	50·8	45·6
			Rolled oats			
548 a	61 ♂	52	7·0	135·4	58·7	43·4
549 b	60 ♀	31	6·9	127·6	58·15	45·5
550 c	61 ♀	45	6·9	148·9	70·8	47·8
551 d	58 ♀	48	6·9	126·7	59·1	46·6
			Whole wheat			
552 a	62 ♂	32	6·9	126·5	56·7	44·8
553 b	60 ♀	29	6·9	131·5	63·7	48·4
554 c	57 ♀	35	6·9	125·1	60·1	48·0
555 d	57 ♀	35	6·9	132·4	62·2	47·0
			Patent flour			
556 a	58 ♂	41	6·9	133·6	53·3	39·9
557 b	56 ♀	36	6·9	126·7	52·5	41·4
558 c	58 ♀	34	6·8	112·1	48·4	43·2
559 d	57 ♀	30	6·9	121·2	52·8	43·6

* Letter exponents refer to litter-mates.

summarised in Table V only the average values for each cereal. Considering the data for extracted femur weights of the three series *in toto*, it is difficult to rank the various cereals in any definite order except to recognise that yellow maize produced the lightest bone.

If we average the weights of ash for our three series of experiments we obtain for maize, oats, wheat, flour and rice respectively 47, 50, 52, 49 and 48 mg., and if we average only the values for Series I and III in which the food consumption was equalised we obtain quite similar values, *viz.* 47, 51, 52, 50 and 48 mg.

Table V. *Average femur analyses.*

Rat femora

Cereal additions	Average dry weight* (mg.)			Average ash weight (mg.)			Average ash %			A/R ratio†		
	I	II	III	I	II	III	I	II	III	I	II	III
Yellow maize	116·3	114·7	106·6	52·6	46·5	43·6	45·2	40·5	40·9	0·83	0·68	0·69
Rolled oats	134·4	117·6	108·5	61·7	47·4	45·3	45·9	40·4	41·7	0·85	0·58	0·72
Whole wheat	128·9	127·4	109·7	60·7	51·2	47·1	47·1	40·3	42·8	0·89	0·67	0·75
Patent flour	123·4	120·9	115·2	51·8	48·9	49·0	42·0	40·5	42·5	0·72	0·67	0·74
Polished rice	—	122·9	114·2	—	51·6	48·4	—	42·0	42·3	—	0·72	0·74

* After fat extraction.

† A, denotes ash weight of a bone "X"; R, the difference in weight between the fat extracted dried bone "X" and its ash.

Calculating the average percentage of ash in a similar manner, we obtain 41, 42, 42, 41 and 42 % respectively in the first case and 42, 43, 44, 42 and 42 % in the second. These differences certainly do not appear impressive.

Blood analyses.

Since Gutman and Franz [1921–22] and Kramer and Howland [1922] showed that either low calcium or low inorganic phosphorus values of blood serum of rats could be used as an index of the severity of rachitic lesions, many investigators have determined these values. Whilst the importance of these as an index is now discounted to some extent [Bethke *et al.*, 1923], and justifiably so, they were nevertheless obtained in our analyses.

Kramer and Howland [1922], McCollum *et al.* [1922, 1], Cavins [1924], Dutcher *et al.* [1925] originally demonstrated with rachitic rats showing subnormal calcium or inorganic phosphate values for blood serum, that starvation results in an increase of these elements in the blood stream. If fasting had occurred it is possible that marked discrepancies would have been introduced into our data. We however need give no consideration to this factor since none of the rats on any of the cereals ever refused food and they were eating daily 9, 6 to 8, and 5 g. respectively at the end of the five weeks of experiment.

The normal calcium values for rat blood serum have been reported by Kramer and Howland [1922], Bethke *et al.* [1923] and by Cavins [1924], as ranging from 9·5 to 10·5, 10·5 to 13·6 and 9·5 to 10·0 mg., respectively, per 100 ml. of serum. The calcium values of the blood sera of all the rats in Series I, II and III, presented in Table VI, are conspicuously below the normal values and range

Table VI. *Calcium and inorganic phosphorus of sera.*

Cereal additions	Ca mg. per 100 ml. serum*			P mg. per 100 ml. serum*			Ca × P per 100 ml. serum*		
	I	II	III	I	II	III	I	II	III
Yellow maize	6·80	4·40	4·28	7·45	8·88	7·25	50·7	39·1	31·0
Rolled oats	5·85	3·80	4·63	8·15	9·04	8·21	47·7	34·4	38·0
Whole wheat	6·60	4·40	4·02	8·10	10·37	8·15	53·5	45·6	32·8
Patent flour	Lost	4·40	3·98	Lost	9·77	9·10	Lost	43·0	36·2
Polished rice	—	4·25	3·94	—	8·65	7·69	—	36·8	30·3

* Sera from pooled bloods.

from a minimum of 3·8 mg., to a maximum of 6·8 mg. per 100 ml. of serum. The rolled oats diets produced the lowest values in Series I and II and the highest in Series III. The calcium values of the remaining cereals compared in each series differ so little that they may be considered identical. In fact, the striking similarity of all these comparative calcium values again points to the approximate equality of these cereals in the production of a mild form of rickets.

The normal inorganic phosphate values for the serum of rat blood have been reported by the same aforementioned authors and by Dutcher et al. [1925] as ranging from 7·0 to 8·5, 9·0 to 9·5, 7·0 to 8·5 and 8·0 to 10·0 mg., respectively, per 100 ml. of serum. The inorganic phosphate values of the sera of Series I, II and III, Table VI, range from 7·25 to 10·37 mg., all of which lie within or slightly above the reported normal ranges. Those for yellow maize and polished rice are the lowest while those for whole wheat and patent flour are the highest.

The separate calcium and inorganic phosphate values of the blood sera also lend themselves to the calculation of additional values which may be used further to compare the cereals. Kramer and Howland [1923] demonstrated that the products of these calcium and inorganic phosphate values in mg. per 100 ml. could be used to indicate the severity of rickets. They concluded that when the product is below 30 rickets is to be expected, when between 30 and 40 it is probable. When above 40 rickets is either healing or entirely absent. The calcium and phosphorus products of Series I, II and III range from 47·7 to 53·5; 34·4 to 45·6; and 30·3 to 38·0, respectively. In Series I and II the largest products are shown by whole wheat and the smallest by rolled oats, but in Series III rolled oats has the largest and both yellow maize and polished rice the smallest values. Since figures obtained as the products of two or more values exaggerate uncontrollable small variations, we believe no significant differences are revealed by these calcium and phosphorus products.

Intake of calcium and phosphorus.

Cereals in general are known to be very low in both calcium and the antirachitic factor and to contain only moderate amounts of phosphorus. Dibbelt [1909] produced rickets in dogs by feeding diets deficient in calcium. Sherman and Pappenheimer [1921] and McCollum, Simmonds and Kinney [1922] produced rachitic bones in rats on diets low in calcium and the antirachitic factor. McCollum et al. [1921, 1, 2; 1922, 2] and Sherman and Pappenheimer emphasised the importance, for normal bone formation, of maintaining a definite relationship between the calcium and phosphorus in the diet. This belief is probably accepted more absolutely than is justifiable because much depends on the amounts of each in the diet [Brown et al., 1932] and as we know now on the form in which they are present [Bruce and Callow, 1934].

Calcium and phosphorus determinations were made on all our rations. The calcium was determined by a volumetric adaptation of the McCrudden method [1911–12] and the phosphorus gravimetrically by precipitation as ammonium phosphomolybdate and magnesium ammonium phosphate. The calcium and phosphorus analyses per 100 g. of ration are shown in Table VII.

The intake of phosphorus per 100 g. of ration is not markedly different from the requirements of 494 mg., originally reported by Shipley [1922] as being normal. It ranged between 70 and 118 % of this value. The rations of yellow maize supplied the requirements; those of rolled oats and whole wheat exceeded these by 18 % ; and those of patent flour and polished rice were too low by 30 %.

The normal amount of dietary calcium required for continuous growth, maintenance and normal function in rats, when all other dietary factors are

Table VII. *Calcium and phosphorus intake and calcium-phosphorus ratios in the rations.*

Cereal additions	Total intake (per rat)						Intake per 100 g. ration					
	Ca (mg.)			P (mg.)			Ca (mg.)		P (mg.)		Ca/P	
	I	II	III	I	II	III	I	II and III	I	II and III	I	II and III
Yellow maize	195	185	143	1196	1268	985	81	66	494	452	1:6·1	1:6·9
Rolled oats	273	231	214	1398	1347	1246	113	99	579	574	1:5·1	1:5·8
Whole wheat	274	283	214	1399	1642	1242	114	99	580	576	1:5·1	1:5·8
Patent flour	210	183	159	839	862	749	87	73	349	344	1:4·0	1:4·7
Polished rice	—	150	130	—	808	699	—	64	—	343	—	1:5·4

supplied, has been reported by Shipley [1922] to be 640 mg. per 100 g. of ration. Casual inspection immediately shows all rations to be low in calcium. They supplied only between 10 and 18 % of the normal amount required, according to this standard. The intakes of this element per 100 g. of ration ranged in Series I from 81 to 114 mg. and in Series II and III from 64 to 99 mg. In the first series approximately 38 % less calcium was furnished by the yellow maize and patent flour rations than by those of rolled oats and whole wheat. In the second and third series alike about 47 % less calcium was supplied by the yellow maize, patent flour and polished rice rations than by those of rolled oats and whole wheat. In all the series the yellow maize rations have the widest ratios, those of patent flour the narrowest and those of polished rice, rolled oats and whole wheat practically identical intermediate values.

SUMMARY.

Rolled oats, patent flour, whole wheat, polished rice and yellow maize when making up 75 % of a low-calcium ration have been compared in their capacity to produce rickets in the rat. The severity of the rachitic lesions produced by these different cereals and cereal products were remarkably similar. In this respect our findings are at variance with those of Green and E. Mellanby [1928] who contend that cereals differ markedly in their capacity to produce rickets, and that oatmeal is the worst offender and white flour the least.

Our conclusions are based on the extent of mineralisation in the distal ends of the radii and ulnae; the degree of hyperplasia of the costochondral junctions; the total weights, ash weights and percentage ash of the fat-extracted femora; and the calcium and inorganic phosphate relations of the blood sera in contrast with the percentage of calcium and the A/R ratio of the leg bones used by Green and Mellanby.

In a previous paper [Steenbock *et al.* 1930] we have presented results which showed that maize, wheat and oats fed with other supplements than those used in these experiments differed somewhat in their bone forming capacities; wheat being the best, oats the next best, and maize the poorest. This brings out the modifying effect of supplements without the use of which the calcifying—or anticalcifying—values of cereals cannot be tested.

It is obvious that generalisations as to the specific bone-building value of different cereals from experimental results with rats are beset with many uncertainties.

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